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# ***Interactive comment on “Using a two-layered sphere model to investigate the impact of gas vacuoles on the inherent optical properties of *M. aeruginosa*” by M. W. Matthews and S. Bernard***

**Anonymous Referee #2**

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## General Comments:

The manuscript reports an optical modeling effort of using two-layered sphere model to simulate the IOPs of vacuolated cyanobacteria *M. aeruginosa*. While layered spherical model has been used to model aquatic particles (e.g., diatom or bubbles), it is new to use it for cyanobacteria with gas vacuoles, with its justification well explained by the authors. This is the innovative part of the manuscript.

Since the mathematical details of the two-layered model are well-known and its programming implementation is already in place, the challenge is really in determining the complex refractive indices as well as the sizes of both the shell (or cyanobacteria) and

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the core. The size distribution of *M. aeruginosa* was represented by log-normal distributions with an effective radius fixed at  $2.58 \mu\text{m}$  and an effective variance varying between 0.01 and 0.05. The size of the core was modeled as volume fraction of the cells. The complex refractive index of the core was determined as volume-weighted mean of the refractive indices of water, air and proteins. The complex refractive index of the cell was determined using the inverse ADA method for  $n'$  and the homogeneous spherical Mie model for  $n$ , and later by fitting the simulated  $\text{Rrs}$  using HydroLight/EcoLight with the observations for a refined  $n$ .

For validation, the authors compared the computed IOPs with published data, particularly those by Zhou et al. (2012) on  $b$  and  $bb$  (and their [Chl] specific values) and by Volten et al. (1998) on VSFs from 20 to 170 deg.

My biggest concern is in the real part of the complex refractive index of the cell, which forms the shell of the two-layered sphere model. Because of the sheer size of the shell (the volume fraction of the core,  $V_g$  mostly varies between 1-10% but was also simulated up to 50%), the real part of the index (the authors used  $1+\varepsilon$  to represent, but it is not exactly the same, it should be  $1+\varepsilon+\Delta n$ , where  $\Delta n$  is computed from  $n'$  based on anomalous dispersion) plays a critical role in determining the IOPs. For example, compare Figs. 4 and 6 to how dramatic changes in IOPs were incurred when  $1+\varepsilon$  varied from 1.08 to 1.036. The inverse ADA relies on the homogeneous spherical Mie model (or its approximation) to estimate the real part refractive index. Also, as authored mentioned the backscattered light was not accounted for in this method, possibly forcing an elevated  $n$  to make up for the lost backscattering. And this might explain why the comparison with Volten et al. (1998) data was very poor in the backward angles.

Using  $\text{Rrs}$  to refine the estimate of  $n$ , in my opinion, raised more questions than answers. In this approach, the authors assumed the variability in observed  $\text{Rrs}$  is entirely due to  $n$  of *M. aeruginosa* cells in a complex water including tripton, CDOM and possibly other particles that were not included in the EcoLight simulation. The  $\text{Rrs}$ -forced  $n$  of the *M. aeruginosa* shell had a value of 1.12, higher than the typical range of the phy-

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toplankton cells. Also, the resultant VSF and  $bb^*$  compared poorly with measurements by Volten et al and Zhou et al, respectively.

Is it possible to use the layered mode in the inverse ADA method directly, at least for the  $Q_c$  part, because the gas vacuoles do not affect  $Q_a$  very much. I believe this could be a significant step forward.

My second major comment is that I found the manuscript difficult to read, not because language but because the structure and some loose use of terms without definitions.

#### Specific Comments.

1. Please clearly define how  $n$  is related to  $1+\varepsilon$ . In ocean optical community, people are more familiar with  $n$ .
2. Is the backscattering probability the same as the backscattering ratio? Can you use the latter term which people are more familiar with.
3. If you use  $m = n + in'$ , then stick with it. For example, P10541 L17, you use  $k$  to represent  $n'$ .
4. Sometimes the  $n$  values are given in vacuum and sometimes in relative to water. It will be easier for readers to follow if used consistently with one convention.
5. P10533 L24-27: "Therefore it appears that vacuoles contained in the cell increase the overall scattering of the cell suspensions equally across the spectrum. This is in contrast to the scattering properties of isolated gas vesicles, which scatter light as Rayleigh scatterers with a  $\lambda^{-4}$  shape." There is nothing wrong with this statement per se, but it is well expected that when individual particles/molecules, same or different, packed together, the scattering changes, e.g., scattering by a water droplet vs. scattering by water molecules.
6. P10549 L507: What do you mean "This effect is probably caused by a breakdown of the assumption of volume-equivalence (Eq. 1) rather than from light shielding or other

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effects."

7. Section 4 Applications. This part makes the paper more relevant to Biogeosciences. However, given the uncertainty associated with modeling IOPs, particularly for the scattering in the backward angles, it is premature to simulate Rrs and to develop algorithms based on the simulated Rrs, which depends strongly on the backscattering. Or the authors might want to evaluate the error in Rrs and associated algorithms given the uncertainty in simulating the backscattering of the vacuolated cyanobacteria cells.

8. References. The pages in the references are messy. For example, the 1st reference "Aas, E.: Refractive index of phytoplankton derived from its metabolite composition, *J. Plankton Res.*, 18, 2223–2249, doi:10.1093/plankt/18.12.2223, 1996. 10536, 10539, 10541, 10554". Also, the reference "Smarda 2009" is out of place.

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